Linkage stratification and mutation analysis at the parkin locus identifies mutation positive Parkinson’s disease families

W C Nichols, N Pankratz, S K Uniacke, M W Pauciulo, C Halter, A Rudolph, P M Conneally, T Foroud, and the Parkinson Study Group

Parkinson’s disease (PD) is one of the most common neurological disorders in humans with an overall prevalence of 1:1000 with the incidence increasing to as high as 3.4% among people aged 75 years. The clinical phenotype includes resting tremor, muscular rigidity, bradykinesia, and postural instability. The signs and symptoms of the disease are the consequence of a striatal deficiency of dopamine resulting from neuronal death in the substantia nigra. It is characterised by the presence of the Lewy body, an intracytoplasmic inclusion body found in many brain regions which is not entirely specific to, but is a highly sensitive marker for, Parkinson’s disease.

The pathogenesis of idiopathic Parkinson’s disease is unknown. For the overwhelming majority of PD patients, the disease has previously been thought to occur sporadically. However, there is increasing evidence of a genetic contribution to the disorder. Recently, two studies have investigated familial aggregation of PD using large, population based, case-control studies. Elbaz et al reported an odds ratio of 3.2 for the presence of PD in first degree relatives (parents and sibs) of 175 cases as compared to 481 controls. Analyses stratified by age showed this aggregation to be stronger for younger PD patients. Familial aggregation of PD in Iceland was studied using a cohort of 772 cases, with 560 having onset of disease before age 50 years. It showed this aggregation to be stronger for younger PD patients. The pathogenesis of idiopathic Parkinson’s disease is unknown. For the overwhelming majority of PD patients, the disease has previously been thought to occur sporadically.

In this study, the odds ratio for PD was 6.7 for sibs and 3.2 for offspring of affected subjects. These studies are consistent with others reporting the risk to be anywhere from two to 14 times higher for first degree relatives as compared to the risk in members of unaffected families. Genetic linkage analyses in families with autosomal dominant forms of Parkinson’s disease, and with the identification of a missense mutation in two PD patients of a German pedigree. Subsequent positional cloning efforts have identified a novel gene, parkin, as the causative gene for autosomal recessive, juvenile parkinsonism. A wide variety of parkin mutations have been identified in families with autosomal recessive parkinsonism and in sporadic cases of Parkinson’s disease of different ethnic origin.

Two additional loci for the autosomal recessive form of the disorder, PARK6 and PARK7, were recently identified on chromosome 1p35-p36 and 1p36, respectively.

**Material and Methods**

In an effort to identify additional genetic factors contributing to Parkinson’s disease, we have initiated the collection and analysis of a large panel of affected sib pairs. Families reporting at least two living sibs diagnosed or considered likely to be diagnosed with PD were ascertained through a variety of sources, including 52 US and Canadian centers participating in the Parkinson Study Group (PSG). All study participants completed a uniform clinical evaluation that consisted of parts II and III of the Unified Parkinson Disease Rating Scale (UPDRS). In addition, a diagnostic checklist was developed based on the results of clinicopathological studies of parkinsonism so as to reach an acceptable degree of diagnostic specificity and sensitivity.

All study participants were then classified as either verified PD (VDP) or non-verified PD (NVPD), based on the results of the diagnostic checklist. The sample to date consists of 230 participants with VDP and 64 participants classified as NVPD giving a total of 162 sib pairs from 148 families and 94 affected sib pairs classified as verified PD from 86 families. Our sample consists of 95% whites and 5% Hispanics with an average age of onset of 60.4 years. Peripheral blood was obtained from all subjects after appropriate written informed consent approved by each individual institution’s IRB. DNA was prepared using standard methods.

As mutations in parkin are the most common inherited defect identified in PD to date, our initial efforts were focused on looking for evidence of linkage to the parkin locus. DNA samples from all 162 sib pairs were genotyped using 21 chromosome 6 dinucleotide repeats which are part of the ABI Prism Linkage Mapping Set (Applied Biosystems, Foster City, CA), including D6S305 located within intron 7 of the parkin gene. Briefly, 30 ng of genomic DNA was PCR amplified using each individual marker in a 10 µl reaction. After PCR, the PCR products were pooled using equal amounts of each PCR reaction. One µl of this multiplexed mix was added to 10 µl formamide containing the GeneScan-400HD ROX size standard (Applied Biosystems, Foster City, CA). Genotypes were determined using the ABI 3700 DNA Analyzer (Applied...
Table 1  Parkin mutations identified in 16 Parkinson disease families

<table>
<thead>
<tr>
<th>Family</th>
<th>No affected</th>
<th>Lod score</th>
<th>Age of onset</th>
<th>Mutation 1</th>
<th>Exon</th>
<th>AA change</th>
<th>Zygosity</th>
<th>Mutation 2</th>
<th>Exon</th>
<th>AA change</th>
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<tr>
<td>33007</td>
<td>2</td>
<td>0.4031</td>
<td>28.44</td>
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<td>7</td>
<td></td>
<td>Hetero</td>
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<td>05007</td>
<td>4</td>
<td>0.7164</td>
<td>45, 45, 45, 45</td>
<td>Deletion</td>
<td>3, 4</td>
<td>Gln57fs [+35 aa]†</td>
<td>Homo</td>
<td>Leu112 fs [+50 aa]</td>
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<td>Deletion</td>
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<tr>
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<td>0.5376</td>
<td>38, 38</td>
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<td>3</td>
<td></td>
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<td>0.1719</td>
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<td></td>
<td>Hetero</td>
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<tr>
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<td>0.3999</td>
<td>24, 30</td>
<td>Deletion</td>
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<td>Gln57fs [+35 aa]</td>
<td>Homo</td>
<td>Arg275 fs</td>
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<tr>
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<td>34, 36</td>
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<td>6</td>
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<td>Homo</td>
<td>Duplication</td>
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</table>

* Nucleotides are numbered according to GenBank Accession AB009973 with the A of the initiator ATG numbered as +1.
† Frameshifts are denoted by the amino acid and its number at which the frameshift occurs as recommended by Dunnen and Antonarakis.

BioSystems, Foster City, CA) and GeneScan 3.5, Genotyper 3.6, and GeneMapper 1.1 software. All genotypic data were checked for Mendelian inheritance of marker alleles with the program Pedcheck. The marker genotypic data, including genotypic data from other parts of the genome, were used to verify the full sib relationships among the subjects using the computer program RELATIVE. Observed allele frequencies in the subjects genotyped for the genome screen were used. Marker order and map positions were obtained from the Marshfield Medical Research Foundation (http://research.marshfieldclinic.org/genetics/). Multipoint affected sib pair linkage analysis was performed using the program Mapmaker/Sibs and included dominance and GeneMapper 1.1 software. All genotypic data were checked for Mendelian inheritance of marker alleles with the program Pedcheck. The marker genotypic data, including genotypic data from other parts of the genome, were used to verify the full sib relationships among the subjects using the computer program RELATIVE. Observed allele frequencies in the subjects genotyped for the genome screen were used. Marker order and map positions were obtained from the Marshfield Medical Research Foundation (http://research.marshfieldclinic.org/genetics/). Multipoint affected sib pair linkage analysis was performed using the program Mapmaker/Sibs and included dominance variance in the model. Lod scores were computed at 1 cM intervals along chromosome 6, using all possible sib pairs formed from families with more than two affected sibs. The parkin gene was previously reported to result in autosomal recessive PD; therefore, parametric linkage analysis was also performed using the computer program Allegro and an autosomal recessive, fully penetrant disease model with the disease allele having a frequency of 0.01. Analyses were performed using two models of affection: (1) including both verified and non-verified PD as affected, and (2) including only verified PD as affected.

Initial analyses of chromosome 6 using all available sib pairs resulted in a maximum lod score of 0.7 at marker D6S305. The highest peak in the region was 30 cM centromeric at D6S292 (lod=1.2). Parametric linkage analyses performed using the program Allegro revealed the presence of a fully penetrant disease allele having a frequency of 0.01. Analyses were performed using two models of affection: (1) including both verified and non-verified PD as affected, and (2) including only verified PD as affected.

RESULTS

Results of the direct sequence and fluorescent dosage analysis are shown in table 1 and fig 1. Seventeen different parkin mutations were identified in 16 of 56 families analysed. Five of the mutations were detected more than once with the missense mutation Arg275Trp (three families) and the exon 8 duplication (3 families) accounting for ~40% of mutation positive families. It is not known whether these recurrent mutations are the result of a shared haplotype or represent independent mutational events. Three (16%) of the mutation positive families are of Hispanic origin; however, none of them carries the same mutation. The remaining 12 mutations were each detected in a single family. Three of the 17 mutations are missense mutations resulting in single amino acid substitutions in the parkin peptide. The remaining 14 are either deletions ranging in size from one base (154delA) up to entire exons or duplications of entire exons. All of the deletion and duplication mutations would predict a frameshift, except for the exon 3 and 4 deletion that predicts an in frame deletion of...
amino acids 58-178 and the exon 5 duplication that predicts an in frame duplication of amino acids 179-206. Affected subjects were determined to be either homozygous or compound heterozygous in nine of the 16 families in which parkin mutations were detected (table 1). Mutations were only detected on one allele in affected subjects in the remaining seven families. Whether these patients are truly heterozygous or compound heterozygotes with the other allele carrying an undetected mutation remains to be determined. Our method of direct sequencing and fluorescent dosage would clearly miss any mutations that are not within the coding region, that is, those in the promoter or introns. Heterozygosity for parkin mutations in PD has been previously reported, and it has also been hypothesised that these subjects probably carry an undetected mutation on their other parkin allele.

**DISCUSSION**

Of the 17 mutations identified in this study, 12 have been previously reported. An additional 116 affected subjects from 58 families who did not have positive parkin lod scores were screened by PCR and restriction endonuclease digestion for some of the mutations reported here as well as other previously reported mutations which were determined to change a restriction site. In this manner, two additional families were identified with mutations, one each with the 219-220insGT mutation previously reported by Abbas et al. and the Gly430Asp mutation reported by Periquet et al. No additional mutations were detected in any other affected subjects/families. Subsequent analyses of only those families without identified parkin mutations resulted in lod scores < 0.1 for this entire region of chromosome 6, suggesting that all families with mutations had been identified.

The approach we are taking to identify additional genetic loci shows the synergy of linkage stratification and direct sequencing to identify mutation positive families. Our initial analysis of either the full sample of 162 affected sib pairs or the subset of 94 sib pairs with verified PD yielded no evidence of linkage to the parkin region. However, stratification of the sample by families with suggestive evidence of allele sharing, or linkage to, the parkin gene enabled the identification of 17 parkin mutations in 16 of 56 (29%) families analysed. While all other reports have stratified samples based on age of onset of PD of 45 years or less for mutation detection, our strategy to analyse those families with evidence of positive lod scores at the parkin locus yielded the highest percentage of parkin mutations of any study to date. The ages of onset of those affected subjects in whom parkin mutations were identified in this study ranged from 22-69 years. However, four of the 16 mutation positive families had ages of onset greater than 45 years for all affected subjects and would have thus been missed if we had used age of onset as our stratification criteria. As well, identification and removal of subjects with mutations in known genes from subsequent genome wide analyses will decrease the heterogeneity in this sample and probably increase the power of this sample of affected sib pairs to detect linkage to additional chromosomal regions harbouring genetic loci contributing to the susceptibility to PD.

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**Figure 1** Location of identified parkin mutations within the parkin gene. The 12 exons of the parkin gene are depicted as rectangles numbered 1-12. Locations of the initiator ATG and terminator TAG are as shown. Missense and small insertion/deletion frameshift mutations are depicted above the gene while mutations resulting in deletions/duplications of entire exons are indicated below the gene. All mutations were identified in a single family except those with a number in parentheses beside the mutation. The number in parentheses indicates the number of families in which those mutations were identified. An asterisk indicates mutations that were detected as heterozygous.
REFERENCES


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